

IN THE CLAIMS

Please amend the claims as follows:

1. (Currently Amended) A method of assaying an enzyme-mediated luminescence reaction comprising:

(a) detecting or determining luminescence energy produced by at least one first enzyme-mediated luminescence reaction which is mediated by an anthozoan luciferase ~~or a peroxidase~~; and

(b) introducing a composition capable of selectively quenching the first enzyme-mediated luminescence reaction and initiating a second enzyme-mediated luminescence reaction distinct from the first enzyme-mediated luminescence reaction, wherein the composition comprises at least one selective quench reagent for the first enzyme-mediated luminescence reaction, wherein the at least one selective quench reagent quenches the first enzyme-mediated luminescence reaction by at least 35-fold; and

(c) detecting or determining luminescence energy produced by the second enzyme-mediated luminescence reaction.

2. (Withdrawn) A method of assaying an enzyme-mediated luminescence reaction comprising:

(a) detecting or determining luminescence energy produced by at least one first enzyme-mediated luminescence reaction; and

(b) quenching photon emission from the first enzyme-mediated luminescence reaction by introducing a composition comprising a colored compound to the luminescence reaction which compound is a selective quench reagent.

3. (Currently Amended) A method of assaying an enzyme-mediated luminescence reaction comprising:

(a) detecting or determining luminescence energy produced by at least one first enzyme-mediated luminescence reaction mediated by an anthozoan luciferase ~~or a peroxidase~~; and

(b) quenching photon emission from the first enzyme-mediated luminescence reaction by introducing a composition comprising at least one selective quench reagent to the luminescence reaction, wherein the at least one selective quench reagent quenches the first enzyme-mediated luminescence reaction by at least 35-fold;

(c) introducing a composition capable of initiating a second enzyme-mediated luminescence reaction distinct from the first enzyme-mediated luminescence reaction; and

(d) detecting or determining luminescence energy produced by the second enzyme-mediated luminescence reaction.

4. (Withdrawn) The method according to claim 2 in which the composition further comprises reagents capable of initiating a second enzyme-mediated luminescence reaction distinct from the first enzyme-mediated luminescence reaction; and

(c) detecting or determining luminescence energy produced by the second enzyme-mediated luminescence reaction.

5. (Withdrawn) The method according to claim 1 or 3 wherein at least one selective quench reagent is a substrate analog inhibitor for the first enzyme.

6. (Original) The method according to claim 1 or 3 wherein at least one selective quench reagent is a sequestering agent.

7. (Original) The method according to claim 6 wherein the sequestering agent sequesters a substrate for the first enzyme but not the second enzyme.

8. (Original) The method according to claim 6 wherein the sequestering agent is a nonionic detergent.

9. (Withdrawn) The method according to claim 6 wherein the sequestering agent is a crown ether, glycol, or cyclodextran.

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10. (Withdrawn) The method according to claim 1 or 3 wherein at least one selective quench reagent is a colored compound.
 11. (Withdrawn) The method according to claim 10 wherein the colored compound quenches blue, green or red light.
 12. (Previously Presented) The method according to claim 1 or 3 wherein in step (a), an anthozoan luciferase-mediated luminescence reaction is detected or determined.
 13. (Canceled)
 14. (Previously Presented) The method according to claim 12 wherein in step (b), the first enzyme-mediated reaction is quenched with a nonionic detergent which is not polyethylene glycol octylphenylether or polyoxyethylene sorbitan monolaurate, a substrate analog inhibitor which is a protected coelenterazine, a yellow compound, or a combination thereof.
 15. (Previously Presented) The method according to claim 14 wherein the luciferase-mediated luminescence reaction is mediated by a native or recombinant *Renilla reniformis* (sea pansy) luciferase.
 16. (Withdrawn) The method according to claim 2 wherein the colored compound quenches blue, green or red light.
 17. (Withdrawn) The method according to claim 2 or 4 wherein in step (a), a luciferase-mediated luminescence reaction is detected or determined.
 18. (Withdrawn) The method according to claim 17 wherein the luciferase-mediated luminescence reaction is mediated by an anthozoan luciferase or a functional equivalent thereof.

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19. (Withdrawn) The method according to claim 18 wherein the luciferase-mediated luminescence reaction is mediated by *Renilla reniformis* (sea pansy) luciferase or a functional equivalent thereof.
20. (Withdrawn) The method according to claim 1, 3 or 4 wherein the second enzyme-mediated luminescence reaction is mediated by an anthozoan luciferase or a functional equivalent thereof.
21. (Withdrawn) The method according to claim 20 wherein the second enzyme-mediated luminescence reaction is mediated by *Renilla reniformis* (sea pansy) luciferase or a functional equivalent thereof.
22. (Original) The method according to claim 1, 3 or 4 wherein the second enzyme-mediated luminescence reaction is mediated by a luciferase.
23. (Previously Presented) The method according to claim 22 wherein the second enzyme-mediated luminescence reaction is mediated by a native or recombinant *Photinus pyralis* (North American firefly) luciferase, or a native or recombinant *Pyrophorous plagiophthalmus* luciferase.
24. (Original) The method according to claim 1, 3 or 4 wherein one of the enzyme-mediated luminescence reactions detects the presence or amount of a substrate, enzyme or cofactor.
25. (Withdrawn/Previously Presented) The method according to claim 1, 3 or 4 wherein in step (a), a peroxidase-mediated luminescence reaction is detected or determined.
26. (Withdrawn) The method according to claim 25 wherein a horseradish peroxidase-mediated luminescence reaction is detected or determined.

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27. (Withdrawn) The method according to claim 1, 2, 3 or 4 wherein in step (a), a phosphatase-mediated luminescence reaction is detected or determined.
28. (Withdrawn) The method according to claim 27 wherein alkaline phosphatase-mediated luminescence reaction is detected or determined.
29. (Withdrawn) The method according to claim 1, 3 or 4 wherein the second enzyme-mediated luminescence reaction is a peroxidase-mediated luminescence reaction.
30. (Withdrawn) The method according to claim 29 wherein the second enzyme-mediated luminescence reaction is a horseradish peroxidase-mediated luminescence reaction.
31. (Withdrawn) The method according to claim 1, 3 or 4 wherein the second enzyme-mediated luminescence reaction is a phosphatase-mediated luminescence reaction.
32. (Withdrawn) The method according to claim 31 wherein the second enzyme-mediated luminescence reaction is an alkaline phosphatase-mediated luminescence reaction.
33. (Previously Presented) The method according to claim 1 or 3 wherein in step (a), an anthozoan luciferase-mediated luminescence reaction is detected or determined; and the second enzyme-mediated luminescence reaction is a second and distinct luciferase-mediated luminescence reaction.
34. (Previously Presented) The method according to claim 33 wherein; and the second enzyme-mediated luminescence reaction is mediated by a native or recombinant beetle luciferase.
35. (Original) The method according to claim 34 wherein the second enzyme-mediated luminescence reaction is mediated by a *Photinus pyralis* or a *Pyrophorus plagiophthalmus* luciferase.

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36. (Original) The method according to claim 34 wherein in step (a), the first enzyme-mediated luminescence reaction is mediated by *Renilla reniformis* luciferase.
37. (Withdrawn) The method according to claim 2 wherein the reaction detects the presence or amount of a substrate, enzyme or cofactor.
38. (Original) The method according to claim 1, 3 or 4 further comprising:
subsequent to detecting or determining luminescence energy produced by the second enzyme-mediated luminescence reaction, quenching the second enzyme-mediated luminescence reaction by introducing a composition comprising at least one second quench reagent capable of quenching the second enzyme-mediated luminescence reaction.
39. (Original) The method of claim 38 wherein the at least one second quench reagent is capable of selectively quenching the second enzyme-mediated reaction.
40. (Previously Presented) The method of claim 2 wherein the selective quench reagent quenches the first enzyme-mediated luminescence reaction by at least 35-fold.
41. (Previously Presented) The method of claim 1 or 3 wherein more than one selective quench reagent is present in the composition.
42. (Original) The method of claim 41 wherein the selective quench reagents quench the first enzyme-mediated luminescence reaction by at least 100-fold.
43. (Currently Amended) An enzyme-mediated luminescence reaction assay kit comprising:
at least one functional enzyme substrate for a molecule to be detected by an anthozoan luciferase- ~~or a peroxidase~~-mediated luminescence reaction;
a suitable first container, the at least one functional enzyme substrate disposed therein;

a composition comprising at least one selective quench reagent for the enzyme which mediates the luminescence reaction, wherein the reagent is a colored compound that quenches red, blue or green light, or is a nonionic detergent which is not polyethylene glycol octylphenylether or polyoxyethylene sorbitan monolaurate;

a suitable second container, the composition disposed therein; and
instructions for use.

44. (Previously Presented) An enzyme-mediated luminescence reaction assay kit comprising:

at least one functional enzyme substrate for a molecule to be detected by the enzyme-mediated luminescence reaction;

a suitable first container, the at least one functional enzyme substrate disposed therein;

a composition comprising at least two selective quench reagents for an anthozoan luciferase, wherein at least one reagent is a nonionic detergent which is not polyethylene glycol octylphenylether or polyoxyethylene sorbitan monolaurate or is a yellow colored compound;

a suitable second container, the composition disposed therein; and
instructions for use.

45. (Withdrawn) The kit according to claim 43 or 44 wherein the selective quench reagent is a substrate analog inhibitor which is a protected coelenterazine.

46. (Withdrawn) The kit according to claim 43 or 44 wherein the selective quench reagent is a crown ether, glycol, or cyclodextran.

47. (Previously Presented) The kit according to claim 44 wherein the selective quench reagent is a nonionic detergent which is not polyethylene glycol octylphenylether-or polyoxyethylene sorbitan monolaurate.

48. (Withdrawn) The kit according to claim 43 or 44 wherein the selective quench reagent is a yellow colored compound.

49. (Previously Presented) A dual reporter enzyme-mediated luminescence reaction assay kit comprising:

- a first functional enzyme substrate for a molecule to be detected by an anthozoan luciferase-mediated luminescence reaction;

- a suitable first container, the first functional enzyme substrate disposed therein;

- a quench-and-activate composition comprising at least two selective quench reagents for an anthozoan luciferase-mediated luminescence reaction and a second and distinct functional enzyme substrate corresponding to a second and distinct enzyme-mediated luminescence reaction, wherein at least two of the selective reagents are selected from a protected coelenterazine, a nonionic detergent which is not polyethylene glycol octylphenylether or polyoxyethylene sorbitan monolaurate and a yellow colored compound;

- a suitable second container, the quench-and-activate composition disposed therein; and instructions for use.

50. (Currently Amended) A dual reporter enzyme-mediated luminescence reaction assay kit comprising:

- a first functional enzyme substrate for a molecule to be detected by a first enzyme-mediated luminescence reaction, wherein the substrate is for an anthozoan luciferase;

- a suitable first container, the first functional enzyme substrate disposed therein;

- a quench-and-activate composition comprising at least three selective quench reagents and a second and distinct functional enzyme substrate corresponding to a second and distinct enzyme-mediated luminescence reaction, wherein at least three of the selective quench reagents are a nonionic detergent that is not polyethylene glycol octylphenylether or polyoxyethylene sorbitan monolaurate, [[and]] a protected coelenterazine that is a substrate analog inhibitor for an anthozoan luciferase, [[is]] and a colored compound;

- a suitable second container, the quench-and-activate composition disposed therein; and instructions for use.

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51. (Previously Presented) The kit according to claim 50 wherein the first functional enzyme substrate, and the second and distinct functional enzyme substrate, are luciferase substrates.
52. (Previously Presented) The kit according to claim 50 wherein the analog-inhibitor is a protected coelenterazine, and the colored compound is a yellow compound.
53. (Withdrawn) The kit according to claim 49 or 50 wherein the sequestering agent is a crown ether, glycol, or cyclodextran.
54. (Original) The kit according to claim 49 or 50 further comprising:
a second quench reagent capable of quenching photon emission from the second and distinct enzyme-mediated reaction; and
a suitable third container, the second quench reagent disposed therein.
55. (Currently Amended) A method of assaying an enzyme-mediated luminescence reaction comprising:
(a) detecting or determining luminescence energy produced by at least one first enzyme-mediated luminescence reaction, wherein the reaction is mediated by an anthozoan luciferase or a peroxidase; and
(b) quenching photon emission from the first enzyme-mediated luminescence reaction by introducing at least one quench reagent to the luminescence reaction, wherein the quench reagent comprises a nonionic detergent that is not polyethylene glycol octylphenylether or polyoxyethylene sorbitan monolaurate, is a yellow colored compound, or a combination thereof.
56. (Currently Amended) A method of assaying an enzyme-mediated luminescence reaction comprising:
(a) detecting or determining luminescence energy produced by at least one first enzyme-mediated luminescence reaction, wherein the reaction is mediated by an anthozoan luciferase or a peroxidase; and

(b) quenching the first enzyme-mediated luminescence reaction by introducing a composition comprising at least one quench reagent to the luminescence reaction, wherein the quench reagent comprises a nonionic detergent that is not polyethylene glycol octylphenylether or polyoxyethylene sorbitan monolaurate, is a yellow colored compound, or a combination thereof.

57-62. (Canceled)

63. (Withdrawn) An enzyme-mediated luminescence reaction assay kit comprising:
at least one functional enzyme substrate for a molecule to be detected by the enzyme-mediated luminescence reaction;
a suitable first container, the at least one functional enzyme substrate disposed therein;
at least one colored compound;
a suitable second container, the at least one colored compound disposed therein; and
instructions for use,

wherein the color of the at least one compound is substantially the same as the light emitted by the enzyme-mediated luminescence reaction.

64. (Withdrawn) An enzyme-mediated luminescence reaction assay kit comprising:
at least one colored compound and at least one functional enzyme substrate for a molecule to be detected by the enzyme-mediated luminescence reaction;
a suitable first container, the at least one colored compound and the at least one functional enzyme substrate disposed therein; and
instructions for use,

wherein the color of the at least one compound is substantially the same as the light emitted by the enzyme-mediated luminescence reaction.

65. (Currently Amended) An enzyme-mediated luminescence reaction assay kit comprising:
a quench-and-activate composition comprising at least one selective quench reagent for an enzyme which mediates a first luminescence reaction and a functional enzyme substrate for a

molecule to be detected by a second and distinct enzyme-mediated luminescence reaction, wherein the enzyme which mediates the first luminescence reaction is an anthozoan luciferase ~~or a peroxidase~~, wherein the at least one selective quench reagent quenches the first luminescence reaction by at least 35-fold, and wherein the selective quench reagent is a nonionic detergent that is not polyethylene glycol octylphenylether or polyoxyethylene sorbitan monolaurate, is a protected coelenterazine that is a substrate analog inhibitor for an anthozoan luciferase, a colored compound that quenches red, blue or green light, or is a combination thereof;

a suitable container, the quench-and-activate composition disposed therein; and instructions for use.

66. (Original) The kit of claim 65 wherein the second enzyme-mediated luminescence reaction is a beetle luciferase-mediated luminescence reaction.

67. (Original) The kit of claim 65 wherein the first enzyme-mediated luminescence reaction is an anthozoan luciferase-mediated luminescence reaction.

68. (Canceled)

69. (Currently Amended) A method of assaying an enzyme-mediated luminescence reaction comprising:

(a) detecting or determining luminescence energy produced by at least one first enzyme-mediated luminescence reaction, wherein the first enzyme is an anthozoan luciferase ~~or a peroxidase~~; and

(b) introducing a composition capable of selectively quenching the first enzyme-mediated luminescence reaction and initiating a second enzyme-mediated luminescence reaction distinct from the first enzyme-mediated luminescence reaction, wherein the composition comprises at least one selective quench reagent which is a protected coelenterazine which is a substrate analog inhibitor for the first enzyme, a nonionic detergent that is not polyethylene glycol octylphenylether or polyoxyethylene sorbitan monolaurate, or is a colored compound that quenches red, blue or green light; and

(c) detecting or determining luminescence energy produced by the second enzyme-mediated luminescence reaction.

70. (Currently Amended) The method of claim 1[,]] or 3 [[or 69]] wherein at least one selective quench reagent is a colored compound that quenches blue, green or red light, a protected coelenterazine which is a substrate analog inhibitor of the anthozoan luciferase, [[is]] or a nonionic detergent that is not polyethylene glycol octylphenylether or polyoxyethylene sorbitan monolaurate.

71. (Currently Amended) The method of claim 1[,]] or 3 [[or 69]] wherein the composition comprises at least two selective quench reagents selected from a colored compound that quenches blue, green or red light, a protected coelenterazine which is a substrate analog inhibitor of the anthozoan luciferase, or a nonionic detergent that is not polyethylene glycol octylphenylether or polyoxyethylene sorbitan monolaurate.

72. (Previously Presented) The method of claim 70 wherein the second enzyme is a beetle luciferase.

73. (Previously Presented) The method of claim 71 wherein the second enzyme is a beetle luciferase.